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# Coordinate regulation of organic osmolytes in renal cells

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**Coordinate regulation of organic osmolytes in renal cells.** Adaptation of cells to prolonged hypertonicity generally involves accumulation of compatible organic osmolytes. Renal medullary cells *in vivo* and in tissue culture accumulate several different organic osmolytes, including sorbitol, inositol, betaine, and glycerophosphocholine (GPC) in response to hypertonicity. For the total concentration of these organic osmolytes to be appropriate for the ambient tonicity, an increase in one should cause the others to fall, minimizing changes in their total concentration. The experiments presented here demonstrate this in tissue culture and investigate the mechanisms involved. Sorbitol is synthesized from glucose, catalyzed by aldose reductase. Betaine is transported into the cells. Hypertonicity increases transcription of the aldose reductase and betaine transporter genes, ultimately elevating cell sorbitol and betaine. If aldose reductase is inhibited, which prevents accumulation of sorbitol, betaine transporter gene expression increases, resulting in a higher cell betaine that compensates for the lower sorbitol. Conversely, when cell betaine is altered by changing its concentration in the medium, aldose reductase transcription changes reciprocally, resulting in compensating changes in cell sorbitol. Hypertonicity increases GPC by inhibiting GPC:choline phosphodiesterase (GPC:PDE), an enzyme that degrades GPC. When cell betaine or inositol is increased by raising its concentration in the medium, GPC:PDE activity rises, reducing cell GPC. Thus, the total of the osmolytes, rather than the level of any individual one, is maintained.

Homer Smith investigated marine organisms in order to better understand the function of mammalian kidneys. The wisdom of this approach has been confirmed repeatedly and is evident in any consideration of the role of compatible organic osmolytes in renal medullas.

It was marine biologists who first elucidated the benefits that accumulation of organic osmolytes confers on cells exposed to hypertonic environments [1]. The same generalizations apply whether the hypertonicity is caused by the high salinity in the ocean or in the renal medullas of concentrating kidneys. The compatible osmolytes hypothesis, as stated by marine biologists, follows from observations in isolated protein systems that polyols and certain amino acids do not significantly perturb protein function over a wide range of concentrations. In contrast, equally high concentrations of NaCl and KCl cause marked perturbation. The hypothesis predicts that by accumulating such organic solutes, rather than NaCl or KCl, cells can safely adapt to hypertonicity. Support comes from numerous observations in a wide variety of osmotically stressed cells. Such cells generally accumulate compatible organic osmolytes rather than NaCl or KCl. The role of these solutes implies that it is their total concentration that counts and not specific properties of the individual solutes.



Five organic osmolytes have been identified in renal medullary cells: glycerophosphocholine (GPC) and inositol [2, 3], sorbitol and betaine [4], and taurine [5]. They apparently serve as compatible solutes whose variable accumulation maintains cell volume and electrolyte content in the face of varying hypertonicity. Their origin and osmotic regulation were deduced from studies of renal cells in tissue culture. Sorbitol is synthesized from glucose catalyzed by aldose reductase. Hypertonicity elevates the activity and abundance of this enzyme [4, 6] by increasing transcription of its gene [7]. Betaine is taken up via a specialized transporter [8]. Hypertonicity raises the number of transporters [8] by increasing their transcription [9]. Osmotic regulation of inositol [10] and taurine [11] uptake also involves increased expression of specific transporter genes [12, 13]. GPC is unique in that its level rises in response to high urea, as well as hypertonicity. GPC accumulation is mainly regulated by changes in its degradation to choline, catalyzed by GPC:choline phosphodiesterase [14, 15].

The main points emphasized in the present presentation are that (1) at a given tonicity, renal cells minimize changes in the total concentration of compatible osmolytes by altering the amounts of the other osmolytes that accumulate when the amount of a particular one is changed [16], and (2) this control occurs via regulation of the same processes that are activated to accumulate the organic osmolytes in response to hypertonicity.

Control of sorbitol and betaine accumulation has been studied in PAP-HT25 cells, which are a continuous line of epithelial cells derived from rabbit renal papilla [17]. Although hypertonicity accelerates transcription of aldose reductase, the enzyme that catalyzes production of sorbitol, the magnitude and duration of increase in transcription depends on how rapidly organic osmolytes, including sorbitol, accumulate [7]. Thus, when accumulation of sorbitol is prevented by inhibiting aldose reductase, hypertonicity increases transcription of aldose reductase to a greater extent and for a much longer time than in the presence of sorbitol synthesis. Similarly, if betaine is increased in the medium (and cells), the elevation of transcription of aldose reductase is greatly attenuated.

The degree to which hypertonicity accelerates expression of the betaine transporter gene similarly depends on how rapidly organic osmolytes accumulate. Betaine transporter mRNA increases more in PAP-HT25 cells when sorbitol accumulation is prevented by inhibiting aldose reductase and increases less when high levels of betaine or inositol are present in the medium and cells [18].

Since GPC accumulation is mainly regulated by activity of GPC:PDE, the enzyme that catalyzes its degradation, the rate of degradation of GPC and the activity of this enzyme were examined as a function of accumulation of inositol and betaine in

MDCK renal cells [19]. High betaine and/or inositol in the medium (and cells) reduces the intracellular GPC level, whether GPC is low, as when the osmolality is normal, or high, as when osmolality is increased by adding NaCl to the medium. Under these conditions degradation of cellular GPC is accelerated and GPC:PDE activity is greatly elevated.

Thus, although the different renal organic osmolytes are chemically distinct and are accumulated by diverse mechanisms, the level of each is affected by the others. A likely explanation is that all respond to a common signal that is unrelated to the specific properties of each. Based on previous studies that related expression of aldose reductase to the sum of the intracellular concentrations of $Na^+$ and $K^+$ and not to cell volume, we have proposed that the signal is intracellular ionic strength [20]. Upon exposure to hypertonicity, before organic osmolytes have accumulated, the intracellular ionic strength should rise in parallel to the osmolality. Then, as organic osmolytes accumulate, the intracellular ionic strength should decrease, diminishing the signal for organic osmolyte accumulation. In this respect, it should make no difference what mix of organic osmolytes is accumulated, only their total concentration. Further, excessive accumulation of any organic osmolyte should decrease the signal for accumulation of all, as observed in the present studies.

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